

Effects of a Specific Supplementation on the Recovery of the Professional Football Player

Efectos de una Suplementación Específica en la Recuperación del Futbolista Profesional

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SUMMARY: During the 38–40 weeks of a championship, a professional football player is confronted with numerous high-intensity training sessions with high physical and mental stress. This situation requires constant adaptation to the training cycles and match schedule. Performance can be quantified by biochemical biomarkers and physiological, morphological and physical parameters. In this study, a specific supplement was developed to improve post-training recovery using components developed to improve the antioxidant, anti-inflammatory and immunological profile. The study was conducted during 4 weeks of competition in an Italian first division football team comprising 28 professional players. Two groups were established, one taking the product (n = 15) and the other taking a placebo (n = 13). Before and after the intervention, biochemical analyses and laboratory tests (dual-energy X-ray absorptiometry) were conducted to monitor changes. The training sessions were controlled with a GPS to monitor the physical load. After the intervention, an improvement in parameters related to iron metabolism was observed. No significant improvements associated with the intervention could be established in body composition values or biomarkers related to oxidative stress or inflammation.

KEY WORDS: Oxidative stress; Inflammation; Biochemical parameters; Iron metabolism; DEXA.

INTRODUCTION

Professional football players have changed their physical condition in recent years to become more competitive. Players manage their lifestyles with a diet and supplementation adapted to the physical demands of training and matches.

A footballer's normal activity during a season occurs over 38–40 weeks and is characterized as a mixed sport with high-intensity acyclic actions (Molina-López *et al.*, 2022).

In the current scenario, improved monitoring of training and matches helps better understand efforts, with the speed of play being around 15 % higher than in recent years (Asimakidis *et al.*, 2024). The distance a footballer typically covers during a match ranges from 9816 to 12277 m, of which 681 to 881 m are at a high speed of around 30 km/h or more (Collins *et al.*, 2021).

The number of matches a professional football team usually plays is 1–3 per week (Djaoui *et al.*, 2017), with many high-intensity actions and accelerations/decelerations followed by short recovery periods (Marqués-Jiménez *et al.*, 2017).

These high physical demands cause changes at the muscular, endocrine and immune system levels, so it is necessary to understand that post-match and post-training recovery strategies are essential (Saidi *et al.*, 2021). Studies of markers, objective and subjective, such as heart rate, have gained special importance based on the rate of perceived exertion, GPS data and saliva and blood analysis (Djaoui *et al.*, 2017).

In addition to fatigue and nutrient deficiency, inadequate post-match recovery can cause alterations in the immune response (Marqués-Jiménez *et al.*, 2017). Thus,

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specific supplements are administered, and the timing, type of food and supplementation administered are important for an effective response (Rojano-Ortega *et al.*, 2021).

Currently, various performance markers are used, including biochemical ones, which enable monitoring of the body's response to training loads (Doeven *et al.*, 2018). Thus, blood data collection is routinely performed in football teams, and a consensus exists on administering essential metabolites/micronutrients such as iron or vitamin D to the player (Collins *et al.*, 2021), since deficiency of both is common among athletes. The main mechanisms by which sport causes iron deficiency are increased demand, high loss and the blockage of absorption due to hepcidin concentrations (Sim *et al.*, 2019).

However, strenuous physical exercise leads to increased production of reactive oxygen species, generating oxidative stress in cellular structures, which negatively impacts performance (Rojano-Ortega *et al.*, 2021). Although supplementation with antioxidants (e.g. vitamin C, vitamin E, and N-acetylcysteine [NAC]) can have positive effects, it has also been observed that high doses of Vitamin C or Vitamin E can be counterproductive, generating a pro-oxidant effect, which can cause muscle damage, decreased performance, poor adaptation to training and perceived muscle pain (Rogers *et al.*, 2023).

The body composition of professional football players has changed over the last few years, with their somatotype migrating in recent decades from an endo-mesomorphic to an ecto-mesomorphic profile (Moya-Amaya *et al.*, 2022). Furthermore, periods of only one month may be sufficient to show changes in the phase angle in relation to cellular health, as well as changes in the lean mass of the lower limbs (Molina-López *et al.*, 2022).

This paper studies the effects of taking a specific recovery compound on parameters related to body composition, oxidative stress or inflammation in a professional football team over a 4-week period during the

competitive season. The hypothesis of this work is that the specific recovery product will produce an improvement in some values related to recovery, oxidative stress or inflammation. However, no major changes were expected due to the period studied.

MATERIAL AND METHOD

Group Selection. All the selected subjects were aged over 18 and belonged to the first team of Udinese Calcio of the Italian football league. The sample comprised 28 players ($n = 28$). No players were excluded during the study, which was conducted between November and December 2023.

Inclusion criteria: Players who are permanent members of the team in any of the playing positions (goalkeepers, defenders, midfielders, forwards, etc.) and are not injured. For this last criterion, it was assumed that the player was available for training and matches during the 4 weeks.

Exclusion criteria: Players awaiting loan, injured players, players removed from the first team and youth players were not selected for the study.

Group Distribution. The groups were randomly assigned:

- Intervention group, which took one dose (one sachet) of the product 4 days per week during 4 weeks. This group comprised 15 players ($n = 15$).
- Control group, which took the placebo product (one sachet) 4 days per week during 4 weeks. This group comprised 13 players ($n = 13$).

Both groups continued with the basic supplementation program pre-established by the club's nutritionists. The supplements selected to produce the basic supplementation program, which the players took before and during the intervention period, were Omega-3, a multivitamin with water-soluble vitamins and minerals, vegetable proteins, creatine, glutamine, Q10, magnesium, probiotics and turmeric.

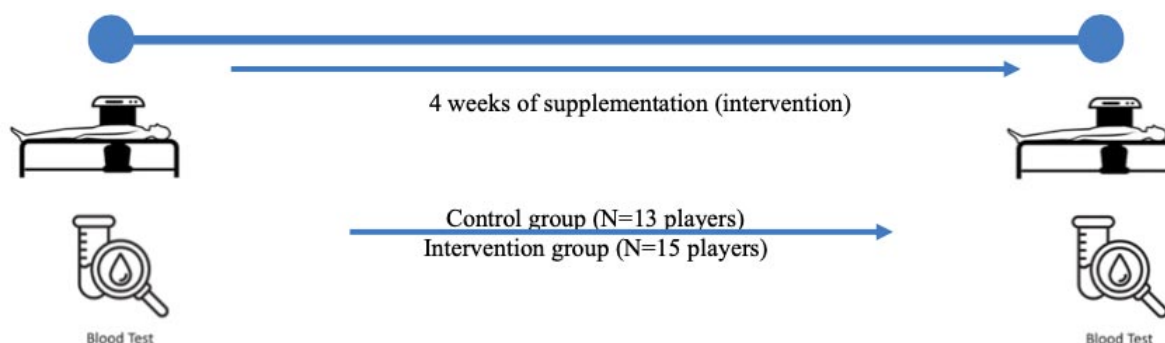


Fig. 1. Study Intervention.

Intervention. An intervention was conducted with a recovery product and a placebo, administered after the post-training meal, in a sample of 28 professional footballers from the Italian first division to observe the possible changes such administration could produce in analytical and morphological parameters. Figure 1 describes the general intervention of the study.

The components and proportions of the product administered in sachets are detailed in Table I. The manufacturing laboratory also produced a placebo product with a similar organoleptic appearance, containing maltodextrin, citric acid, flavorings, silicon dioxide, coloring and sweetener. Both products were administered dissolved in 250 ml of pineapple juice. Laboratory analyses were conducted to ensure that the product was free of doping substances.

Table I. Composition of the intervention product.

CONTENT	PER ENVELOPE (DAILY DOSE)
L-glutamine	2000mg
Bromelain 2500 GDU*/g	500 mg (1250 GDU)
Montmorency cherry concentrate	480 mg
N-acetylcysteine	300 mg
Vitamin C	200 mg
Quercetin	50mg
Zinc	10mg
Selenium	55µg

*The GDU or 'gelatin digestion unit' is a measurement used to quantify the activity of the enzyme bromelain, which is found in pineapple and has proteolytic properties, meaning it can break down proteins.

Test. At the beginning of the study, the Friuli Coram laboratory in Udine (Italy) performed a complete blood analysis, with antioxidant, inflammatory, hormonal, biochemical, blood count and mineral profiles.

A full-body dual-energy X-ray absorptiometry (DEXA) was conducted to measure body composition parameters (GE Healthcare Lunar iDXA model, Madison, WI, USA), evaluating total lean mass, fat mass and its percentage and lean mass in the lower limbs.

This was followed by a 4-week intervention period, after which blood tests were repeated in the same laboratory and the DEXA study was performed under the same conditions as in the first intervention.

Samples were always taken in the morning, between 8 and 10 a.m., after an overnight fast of at least 8 hours. Blood was drawn from the right arm at the level of the elbow flexure, from the antecubital vein, with the subject seated.

This study was approved by an ethics committee and conducted in accordance with the Codes of Ethics for Research Involving Human Beings of the World Medical Association (Declaration of Helsinki), approved by the Junta de Andalucía with internal code: 2061-N-21 and presented in certificate CEI VM-VR_06/2021/N. The athletes signed an informed consent form explaining the purpose and protocol of the study.

Statistical Analysis. The means and standard deviations of all the parameters analyzed were calculated. Shapiro-Wilk tests were performed to check the normality of the variables and, since this condition was always met, the Student's t-test was performed to observe whether significant differences existed in the variables studied.

The variations from pretest to post-test were then calculated for all players, and the means and standard deviations of these variations were obtained. Student's t-tests were performed again to determine whether significant differences existed in these variations, which we estimated would be due to the supplementation administered. The effect size was also calculated using Cohen's d.

Effect sizes were interpreted according to Cohen (1988): minimal effect (<0.20), small effect ($0.20-0.50$), moderate effect ($0.50-0.80$) or large effect (>0.80). If differences are significant ($p < 0.1$) and the effect size is moderate or large, supplementation is indeed effective. If differences are quasi-significant ($0.05 < p < 0.1$) and the effect size is moderate or large, this may indicate that the intervention is effective but was too short or the number of subjects in each group was too small and longer interventions or more subjects would have made a difference. This is also interpreted as a positive result.

Additionally, the pretest to post-test variations of both groups were pooled and Pearson correlation coefficients between the study variables were determined. The following thresholds were used: trivial (<0.1), weak ($0.1-0.3$), moderate ($0.3-0.5$), strong ($0.5-0.7$), very strong ($0.7-0.9$) and almost perfect (>0.9) (Hopkins *et al.*, 2009).

RESULTS

Among all the biochemical parameters measured, we selected those that could be related to the administration of the proposed product by effect size. We present these in Table II. Most of these variables, except calcium, are related to iron metabolism or the immune system. All effect sizes are moderate to high.

Table II. Descriptive statistics of the study variables. Values before the intervention, changes after the intervention and effect sizes when comparing the changes between the groups.

Variables	Experimental group (n = 15)		Control group (n = 13)		Differences	
	Pretest Average \pm sd	Changes Average \pm sd	Pretest Average \pm sd	Changes Average \pm sd	p-value	Cohen's d
Red blood cells ** $\times 10^6/\mu\text{L}$	5.06 \pm 0.20	0.06 \pm 0.22†	5.08 \pm 0.35	-0.11 \pm 0.27†	0.074	0.70
Haemoglobin** g/dL	15.08 \pm 0.90	0.29 \pm 0.67†	14.98 \pm 0.90	-0.20 \pm 0.73†	0.073	0.71
Haematocrit** expressed as %	45.49 \pm 2.46	0.47 \pm 1.75†	45.38 \pm 2.48	-0.91 \pm 1.98†	0.061	0.74
Neutrophil granulocytes * $\times 10^3/\mu\text{L}$	2.22 \pm 0.53	-0.05 \pm 0.049#	2.33 \pm 1.04	-0.64 \pm 0.74#	0.020	0.93
Basophil granulocytes** $\times 10^3/\mu\text{L}$	0.013 \pm 0.035	-0.007 \pm 0.026†	0.015 \pm 0.038	0.015 \pm 0.038†	0.079	-0.69
Lymphocytes* expressed in %	43.46 \pm 7.56	-0.82 \pm 5.44#	46.14 \pm 8.45	4.47 \pm 7.54#	0.041	-0.80
Total iron ** $\mu\text{g/dL}$	85.00 \pm 22.60	20.80 \pm 44.79†	94.46 \pm 28.34	-8.69 \pm 32.87†	0.061	0.75
Calcium * mg/dL	9.81 \pm 0.21	0.17 \pm 0.26#	9.88 \pm 0.41	-0.12 \pm 0.40#	0.033	0.84
Transferrin * mg/dL	247.47 \pm 16.50	8.00 \pm 16.31##	251.15 \pm 30.44	-9.69 \pm 13.37##	0.005	1.19
Saturated transferrin ** mg/dL	68.00 \pm 18.08	16.64 \pm 35.83†	75.57 \pm 22.68	-6.95 \pm 26.30†	0.061	0.75
T3 free** pmol/L	5.50 \pm 0.44	0.16 \pm 0.32†	5.71 \pm 0.50	-0.06 \pm 0.36†	0.087	0.67

*Significant differences between the groups before the intervention ($p < 0.05$); **Significant differences between the groups before the intervention ($p < 0.01$); †Quasi-significant differences in the changes experienced by the groups after the intervention ($0.05 < p < 0.10$); #Significant differences in the changes experienced by the groups after the intervention ($p < 0.05$); ##Significant differences in the changes experienced by the groups after the intervention ($p < 0.01$).

Regarding the variables with a statistically significant variation, we highlight transferrin, calcium, lymphocytes and neutrophils, although parameters such as haemoglobin presented almost significant variations.

The DEXA values obtained in the evaluations indicate that during the intervention period, the players improved their body composition in terms of reduction of fat mass and increases in lean mass in the lower limbs, although in neither case significantly, as shown in Table III. Table IV shows the main parameters related to the antioxidant effect of the product.

Significant and strong correlations were identified between calcium variations and various haematological parameters, including variations in the number of red blood cells ($r = 0.589$; $p = 0.001$), haemoglobin concentration ($r = 0.595$; $p = 0.001$), haematocrit ($r = 0.589$; $p = 0.001$) and total iron ($r = 0.570$; $p = 0.002$). Likewise, significant correlations were found between transferrin variations and red blood cells ($r = 0.506$; $p = 0.006$), haemoglobin ($r = 0.530$; $p = 0.004$), haematocrit ($r = 0.581$; $p = 0.001$) and total iron ($r = 0.519$; $p = 0.005$). Furthermore, a very strong correlation was observed between calcium and transferrin variations ($r = 0.620$; $p < 0.001$). Figure 2 shows the highly

Table III. Descriptive statistics of the study variables measured by DEXA. Values before the intervention, changes after the intervention and effect sizes when comparing the changes between the groups.

Variables	Experimental group (n = 15)		Control group (n = 13)		Differences	
	Pretest Mean \pm sd	Changes Mean \pm sd	Pretest Mean \pm sd	Changes Mean \pm sd	p-value	Cohen's d
Total lean mass kg	67.10 \pm 17.98	3.53 \pm 14.91	71.00 \pm 9.05	-0.92 \pm 1.46	0.315	0.42
Fat mass kg	9.46 \pm 1.61	-0.31 \pm 0.36	9.94 \pm 2.89	-0.63 \pm 0.65	0.124	0.61
Fat mass %	11.35 \pm 2.16	-0.29 \pm 0.52	11.64 \pm 2.52	-0.52 \pm 0.61	0.321	0.40
Lean mass in lower limbs kg	26.79 \pm 2.96	0.37 \pm 0.38	27.27 \pm 4.42	0.19 \pm 0.58	0.358	0.36

significant correlations, highlighting very strong and close to perfect relationships between the variables analyzed.

Table IV. Parameters related to the antioxidant effect. Values before the intervention, changes after the intervention and effect sizes when comparing the changes between the groups.

Variables	Experimental group (n = 15)		Control group (n = 13)		Differences	
	Pretest Mean \pm sd	Changes Mean \pm sd	Pretest Mean \pm sd	Changes Mean \pm sd	p-value	Cohen's d
Biological potential						
Antioxidant $\mu\text{mol/L}$	2365.07 \pm 108.05	27.53 \pm 125.46	2367.77 \pm 153.90	57.92 \pm 172.88	0.596	-0.20
Reactive oxygen metabolites U CARR						
	344.73 \pm 54.50	51.53 \pm 71.42	334.40 \pm 48.89	76.31 \pm 65.47	0.350	-0.36
Serum zinc $\mu\text{g/dl}$						
	81.00 \pm 10.30	12.93 \pm 10.98	75.23 \pm 13.26	15.85 \pm 15.78	0.571	-0.21
Q10 $\mu\text{g/L}$						
	494.77 \pm 127.59	348.36 \pm 321.20	518.43 \pm 182.61	265.65 \pm 358.29	0.525	-0.21

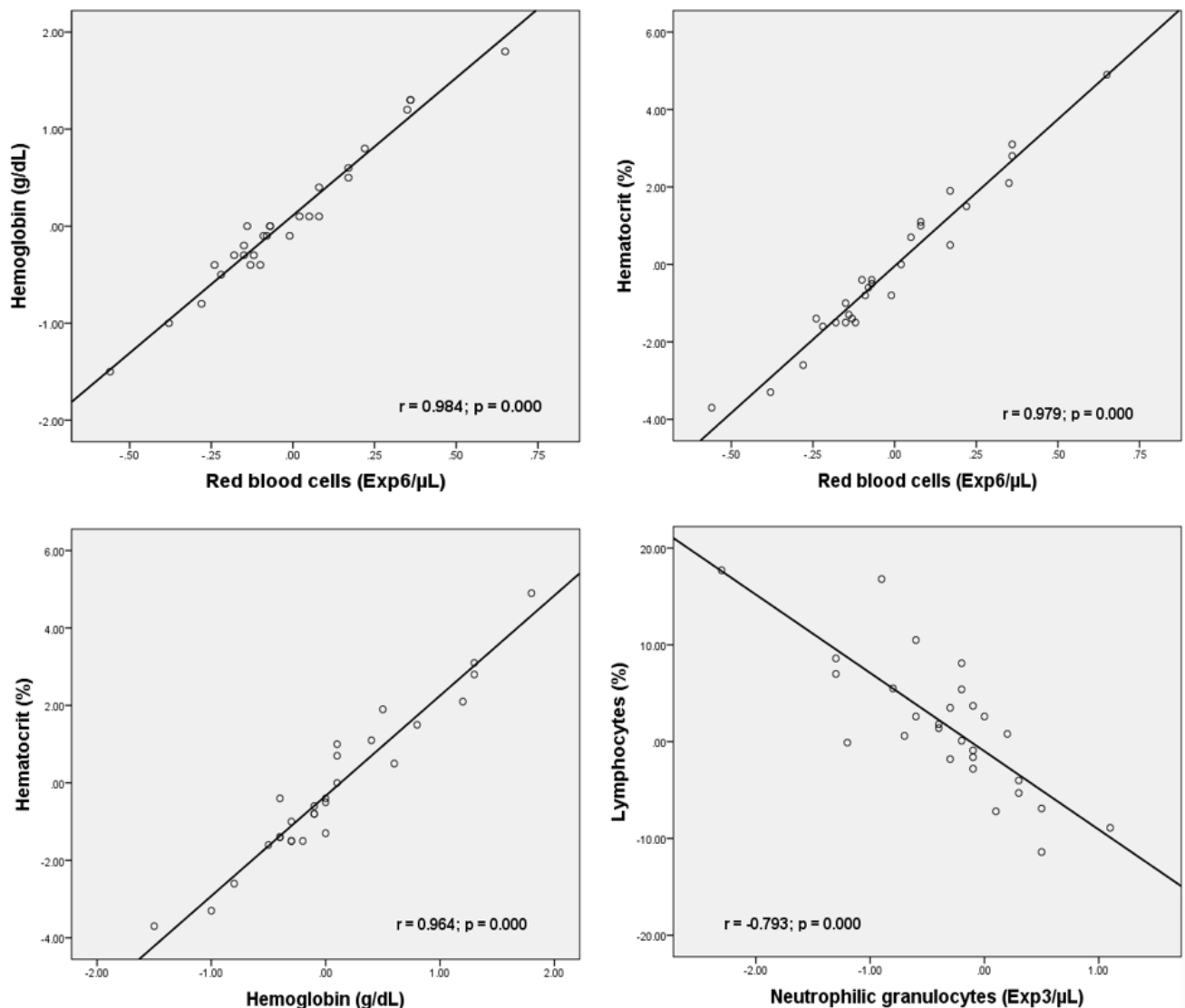


Fig. 2. Significant correlations of the variables studied.

DISCUSSION

In this study, a significant improvement in iron metabolism-related variables, such as haemoglobin, haematocrit and serum iron levels, was observed after specific supplementation. These results underline the crucial role of personalized nutritional strategies in the recovery and performance of professional footballers.

Recent studies reinforce that iron metabolism in athletes can be compromised by high physiological demand, exercise-induced losses and low-grade inflammation generated by high-intensity activities. Peeling & McKay (2023), highlight that subclinical inflammation and increased hepcidin levels decrease iron bioavailability, negatively affecting oxygen transport and aerobic performance. Consistent with our findings, supplementation with antioxidants such as NAC and quercetin appears to have a positive effect by mitigating inflammation and optimizing circulating iron levels.

Tavares-Silva *et al.* (2021), link inflammatory status and iron metabolism. They suggest that underlying mechanisms involving low-grade subclinical chronic inflammation along with changes in hepcidin levels affect iron metabolism, resulting in lower serum iron levels.

The benefits of supplementation in athletes are not limited to iron metabolism. Fernández-Lázaro *et al.* (2022), observed that athletes undergoing hypoxic training programs showed significant improvements in haematological parameters, such as erythropoietin and reticulocytes, as well as an increase in haemoglobin and haematocrit. Although our study did not include specific training interventions such as the one mentioned above, the results reflect similar improvements in the same haematological parameters due to nutritional intervention, which reinforces the effectiveness of supplementation in optimizing oxygen transport and improving physical performance.

Likewise, Rago *et al.* (2022), highlight that accumulations of intense loads during training and competitions can compromise iron stores in elite athletes. In our study, the positive correlation between transferrin and parameters such as haematocrit and haemoglobin supports this relationship, underlining the importance of monitoring and correcting iron levels during intense competitive periods.

The timing of supplementation is also key to its efficacy. Peeling & McKay (2023), recommend administering iron supplements immediately after exercise to exploit lower hepcidin levels and maximize intestinal absorption. Additionally, combining iron intake with sources

of vitamin C further enhances its absorption. These strategies, although not specifically addressed in our protocol, could be integrated into future studies to improve the results obtained. Nevertheless, the athletes had a balanced diet complementary to the nutritional protocol under study.

L-glutamine supplementation for 3 weeks significantly increases immunoglobulin A and nitric oxide concentrations in saliva, reducing the incidence of upper respiratory tract infections in athletes, according to Lu *et al.* (2024). However, we did not observe these effects in our intervention, although we observed improvements in other immunological parameters, such as an increase in neutrophil granulocytes.

Grabs *et al.* (2017), observed that in vitro treatment with bromelain selectively eliminates molecules from the cell surface that can affect the migration and activation of lymphocytes and neutrophils, which could partly explain the anti-inflammatory influence. We did not observe these improvements in our intervention, although the dosages and number of doses in our case differed.

Authors such as Yavari *et al.* (2015), consider that the intake of antioxidants in the diet is insufficient and that additional antioxidants are needed. Studies on young elite footballers have concluded that, after training or during competition, an excess of free radicals is produced and, therefore, oxidative stress, which could reduce the effectiveness of the antioxidant system (Djordjevic *et al.*, 2012). Therefore, from a preventive viewpoint, a diet rich in antioxidants such as fruits and vegetables is recommended (Jayedi *et al.*, 2018).

Supplementation with tart cherry concentrate in intermittent sports such as football, in which inflammatory and oxidative stress markers were measured, is a suitable tool to optimize and accelerate recovery in athletes when ingested days before and several days after a competition (Rojano-Ortega *et al.*, 2021).

Other interesting compounds are available, such as antioxidant supplementation with quercetin and NAC to improve training parameters, which positively affect recovery, adaptation and performance in the athlete population (Lamprecht 2015).

In a study of non-professional triathletes, Riva *et al.* (2018), found that quercetin administration improves recovery speed and performance-related parameters. Particularly, it improves recovery time. In our study, at lower doses, we

observed improvements in parameters such as haemoglobin and haematocrit, which are related to better recovery and performance capacity. Although the antioxidant effects of the athletes' aerobic exercise did not reach statistical significance, they probably contributed indirectly to the modulation of inflammation and the improvement of iron metabolism.

We observed no significant differences in the antioxidant effect indicators, measured through antioxidant biological potential and reactive oxygen metabolites, between the groups subjected to the intervention with the product and the placebo control (Table IV). In 2020, Fernández-Lázaro *et al.* (2020) associated selenium supplementation with an increase in endogenous antioxidant capacity; however, the time required to observe this effect was not specified, which hinders direct comparison with our findings, where this benefit was not detected.

However, the increases in calcium levels observed in our sample could be associated with an improvement in muscle contraction, since continuous training contributes to optimizing muscle function, which increases the demand for calcium to support contractile function (Snijders *et al.*, 2019). Nevertheless, it is notable that in our intervention, calcium was not administered through the supplement; thus, this increase could be due to factors inherent to the training and not to the direct calcium administration.

CONCLUSIONS

We observed an improvement in some variables related to iron metabolism. This can be explained by the supplement containing components that could improve iron absorption in the intestine and protect against the degradation of red blood cells.

Improving parameters related to iron metabolism, especially haematocrit and haemoglobin, can positively impact physical performance, especially in endurance activities such as running, swimming or cycling, as they improve oxygen transport.

Most of the compounds in the proposed supplement stimulate the immune system, which may explain the increase in neutrophil granulocytes.

Although most of the compounds present in the supplement have antioxidant or anti-inflammatory properties, no significant improvement occurred in the parameters evaluated in this regard. It would be interesting in future research to evaluate this effect more precisely by modifying the doses of the components, the posology or the intervention time.

Although the intervention had a positive impact, the integration of additional strategies, such as optimizing the timing of administration and combining it with specific training, could further enhance the results.

Limitations and Future Proposals

Although the results are encouraging, the short duration of the study may have limited the observation of more significant changes in antioxidant parameters and body composition.

During high performance, it is difficult to maintain a placebo intervention for a long period throughout a competition.

Additionally, the lack of control over dietary antioxidant intake and strength training performed in the gym could have influenced the final results. In future studies, it would be useful to consider the following:

- Longer interventions extend the supplementation period to evaluate long-term effects on antioxidant, inflammatory and metabolic parameters.
- Stricter dietary control: monitoring and standardizing dietary intake of antioxidants and other essential nutrients.
- Incorporation of specific training protocols, such as altitude or hypoxic training, to compare their impact on iron metabolism with that of supplementation.
- Adjusted doses to evaluate higher doses of key antioxidants and their effect on parameters of oxidative stress and inflammation.

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RESUMEN: Un jugador de fútbol profesional durante las 38-40 semanas de un campeonato se enfrenta a numerosas sesiones de entrenamiento de alta intensidad, con un elevado estrés físico y mental. Esta situación requiere un proceso de adaptación constante a los ciclos de entrenamiento y al calendario de partidos. El rendimiento puede cuantificarse mediante biomarcadores bioquímicos y parámetros fisiológicos, morfológicos y físicos. En este estudio, se ha administrado después de los entrenamientos, un suplemento específico para mejorar la recuperación, utilizando compuestos destinados a mejorar el perfil antioxidante, antiinflamatorio e inmunológico. El estudio se llevó a cabo durante cuatro semanas de competición en un equipo de fútbol italiano de primera división compuesto por 28 jugadores profesionales. Se establecieron dos grupos, uno que tomaba el producto (n=15) y otro que tomaba un placebo (n=13). Antes y después de la

intervención, se realizaron análisis bioquímicos y pruebas de laboratorio (DEXA) para controlar los cambios. Las sesiones de entrenamiento se controlaron mediante un sistema GPS para monitorizar la carga física. Tras la intervención, se observó una mejora de los parámetros relacionados con el metabolismo del hierro. No se pudieron establecer mejoras significativas asociadas a la intervención en los valores de composición corporal, ni en los biomarcadores relacionados con el estrés oxidativo y/o la inflamación.

PALABRAS CLAVE: Estrés oxidativo; Inflamación; Antioxidante; Hierro; DEXA.

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